

Diarrhea in Children under 5 Years of Age from Ifakara, Tanzania: a Case-Control Study

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A matched case-control study was conducted in the Maternal and Child Health Clinic (MCH) in Ifakara, Tanzania, during the rainy season in order to elucidate the risk factors for and etiology of diarrheal diseases in children under 5 years of age. Cases (103) and controls (206) were matched for sex and age group. Precoded questionnaires with demographic details, clinical history, and physical signs were completed. Stools samples were collected for bacterial, parasitological, and viral studies. A high number of siblings (odds ratio [OR], 0.86; $P = 0.027$), the number of siblings surviving (OR, 0.82; $P = 0.007$), the birth order (OR, 0.85; $P = 0.018$) and the distance from the house to the water source (OR, 0.33; $P = 0.011$) were associated with the risk of diarrhea. There were high rates of enteropathogen isolates in stool samples from children without diarrhea (52.23%). *Shigella* species were the only enteropathogen statistically related with diarrhea (OR, 2.90; $P < 0.029$). Enterotoxigenic, enteropathogenic, and enteroaggregative strains of *Escherichia coli* were not related with diarrhea, and neither were *Giardia lamblia* or *Salmonella* species.

Diarrhoeal diseases are a leading cause of morbidity and mortality among young children in low-income countries (13, 18). Although oral rehydration has been shown to reduce early child mortality, the diarrhea-specific mortality in children less than 5 years of age in Africa has been estimated at about 10.6 per 1,000 (18). At St. Francis Designated District Hospital, diarrhea is the fourth most common diagnosis in inpatients and outpatients and the fourth most common cause of death in admitted children (D. Schellenberg, personal communication).

Causes of diarrhea in areas of endemicity include a wide variety of bacteria, viruses, and protozoa. Poor food hygiene, water, and sanitation are common in communities with high levels of diarrheal disease. Underlying conditions, such as malnutrition, which modify the risk of contracting diarrhea, are also common in much of sub-Saharan Africa. These factors combine to facilitate the spread of enteropathogens, and epidemics are common in such settings.

Apart from well-described enteropathogens such *Shigella* spp., *Salmonella* spp. or enterotoxigenic *Escherichia coli* (ETEC), there are a number of other organisms, such as enteroaggregative *E. coli* (EAggEC) for which the link with diarrheal disease is controversial (2, 13, 15, 18).

We conducted a matched case-control study in order to elucidate the risk factors for and etiology of diarrheal disease in children under 5 years of age in Ifakara, Tanzania. Particular attention was paid to identifying different strains of *E. coli* in order to assess their pathogenicity in this population.

Study area, cases, and controls. The study was carried out in the town of Ifakara, Kilombero District, in the southwest part of Tanzania. The town has 40,000 inhabitants, the majority of whom are small-scale farmers. The area has two wet seasons (the main rains from March to May and the short rains from

December to January). Because during the rainy season, bacteria are more prevalent and the cases of diarrhea increase, the study was conducted during the main rainy season in 1997.

Patients were children under 5 with acute diarrhea, defined by the passing of three or more loose or watery stools in the 24-h period prior to presentation. Controls were healthy children who did not fulfill this case definition during the 2 weeks preceding entry to the study. Two controls were selected for each case recruited at the Maternal and Child Health Clinic in Ifakara. Cases and controls were matched for sex and age group. The age groups defined were <1 year, 1 to 3 years, and 4 to 5 years. Children having taken antibiotics in the previous 2 weeks were rejected for the study.

Precoded questionnaires were completed at the time of recruitment to record demographic details, the clinical history, and physical signs, and a stool sample was collected from all children and processed for bacterial, parasitological, and viral studies in Ifakara. Isolates of *E. coli* were stored for subsequent identification of virulence factors in the Laboratory of Microbiology of Hospital Clínic, Barcelona, Spain. Diarrhea patients were treated with oral rehydration therapy and, where necessary, appropriate antibiotics selected on the basis of the culture results.

Laboratory methods. Laboratory technicians were not given any clinical information, and all samples were processed as follows.

(i) Parasitological studies. Fresh specimens were examined directly for vegetative forms of parasites. The visualized amoebic cysts were confirmed by Heidehain staining. Stools were examined after concentration by the merthiolate-iodine-Formalin technique and were stained with Kinyoun's carbol-fuchsin.

(ii) Bacteriological studies. Feces were inoculated on blood, *Salmonella-Shigella*, MacConkey, cefsulodin-Irgasan-novobiocin, and thiosulphate-citrate-bile salts-sucrose agars. They were incubated at 37°C for 24 to 48 h. For *Salmonella* enrichment, feces were inoculated in Selenite-F broth, incu-

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bated at 37°C for 18 h, and subcultured on *Salmonella-Shigella* agar. All plates were examined, and the colonies suspected of corresponding to enteropathogenic bacteria were identified by standard microbiological methods and commercial antisera. *Yersinia* species were identified after inoculation of stool in 10 ml of tryptose broth and incubation for 3 weeks at 4°C with subsequent subculture on cefsulodin-Irgasan-novobiocin agar. The *Campylobacter* blood-free medium was used to isolate *Campylobacter* species and was incubated for 48 h at 42°C under microaerophilic conditions.

Stools were screened for enterohemorrhagic *E. coli* by plating on sorbitol-MacConkey agar. All non-sorbitol-fermenting colonies were tested in an agglutinating assay with O157 and H7 antisera. Two colonies of each morphologically different type of *E. coli* strains were studied by PCR techniques to detect plasmid DNA for EA_gEC and the *ipaH* chromosomal gene for enteroinvasive *E. coli* strains; enteroaggregative stable toxin type 1 (EAST1 gene); VT1 and VT2 genes for verotoxigenic *E. coli* strains; heat-stable (ST) and heat-labile (LT) enterotoxins for ETEC strains; and the *eae* gene and *bfp* gene and enteropathogen adherence factor (EAF) for enteropathogenic *E. coli* (EPEC) strains. EPEC strains were considered positive when at least one of these characteristics were found. The conditions used for the PCR technique were those described elsewhere (20).

Data management and statistical methods. All data were double entered into Froxpo databases and locked after checks for internal and external consistency. It was estimated that a sample size of 100 cases and 200 controls would have 80% power to detect a doubling in risk of symptoms associated with infection with an enteropathogen, assuming a prevalence of 20% in controls and a 5% significance level. Conditional logistic regression was used to evaluate how the risk of having a case of disease varied for different risk factors and pathogens. Forward stepwise regression was used to select independent risk factors which were then used to adjust estimates of risk of symptoms with individual enteropathogens. The analysis was performed using Stata statistical software (release 5.0; Stata Corporation, College Station, Tex.).

RESULTS

A total of 103 cases and 206 matched controls were recruited. Baseline data are shown in Table 1.

Risk factors for diarrhea. We have complete information on all risk factors for 233 children. In the univariate analysis, a high number of siblings was associated with a reduced risk of diarrhea (odds ratio [OR], 0.86 [0.75 to 0.98]; $P = 0.027$), as well as the number of surviving siblings (OR, 0.82 [0.71 to 0.94]; $P = 0.007$). Birth order was also associated with risk of diarrhea (OR, 0.85 [0.74 to 0.97]; $P = 0.018$), with the late born scoring the lower risk for diarrhea.

Increasing the time taken to reach the water source was also associated with a lower risk of diarrhea (P for linear trend = 0.02), with the risk of diarrhea being much less if water more than 10 min away from the house than if it was less than 1 min away (OR, 0.33 [0.14 to 0.77]; $P = 0.011$). Most children (185) had a mixed diet (traditional weaning food plus breastfeeding). Only four children took breast-feeding exclusively, and all of these were controls. There were no significant differences concerning the risk of diarrhea between children receiving breast milk and those who were being weaned.

Having a latrine within the compound was associated with lower odds of having a case of disease (OR, 0.40 [0.16 to 0.94]; $P = 0.037$). Out of 307 latrines, 278 (91%) latrines were simple

TABLE 1. Baseline data

| Variable | Study group | |
|--|-------------|-----------|
| | Control | Case |
| Mean birth weight (kg) (SD) | 3 (2.26) | 2.8 (0.5) |
| % Muslim | 90 (45.2) | 43 (45.3) |
| No. (%) being in shamba 7 days before | 32 (15.8) | 23 (22) |
| No. (%) receiving breast-feed | | |
| Exclusively | 4 (2.1) | 0 |
| Not at all | 71 (37.4) | 32 (31.4) |
| Mixed | 115 (60.5) | 70 (68.6) |
| Mean age (yr) (SD) | 1.9 (1.2) | 1.6 (1.0) |
| Mean no. of siblings (SD) | 2.1 (2.3) | 1.6 (1.7) |
| Mean no. of siblings alive (SD) | 2.1 (2.2) | 1.4 (1.5) |
| Mean birth order (SD) | 3.0 (2.2) | 2.5 (1.6) |
| Mean time in career school (SD) | 5.5 (2.8) | 5.8 (2.6) |
| No. (%) mud house | 67 (32.5) | 39 (38.2) |
| No. (%) iron roof | 104 (50.5) | 58 (38.2) |
| No. with water source (%) | | |
| Tap | 27 (13.1) | 18 (18.6) |
| Covered | 173 (84) | 80 (78) |
| Uncovered | 5 (2.4) | 2 (2) |
| River | 1 (0.5) | 1 (1) |
| Time (min) from water source | | |
| <1 | 16 (7.8) | 16 (15.7) |
| 1–10 | 114 (55.3) | 59 (57.8) |
| >10 min | 76 (36.9) | 27 (26.5) |
| No. (%) boiled drinking water | 17 (8.3) | 6 (5.9) |
| No. (%) filtered drinking water | 6 (2.9) | 3 (2.9) |
| No. (%) with latrine in house | 196 (95.2) | 90 (88.2) |
| Mean no. (%) of other users of latrine | 1.2 (0.5) | 1.3 (0.6) |
| No. (%) latrine type | | |
| Simple | 190 (92.7) | 88 (86.3) |
| Covered | 38 (18.5) | 23 (22.8) |
| No. (%) visible stool | | |
| Not in latrine | 177 (87.6) | 92 (90.2) |
| Around latrine | 6 (3) | 2 (2) |
| Within latrine | 19 (9.4) | 8 (7.8) |
| Age group (yr) | | |
| 0–1 | 8 (3.9) | 4 (3.9) |
| 1–3 | 134 (65.1) | 67 (65.1) |
| 4–5 | 69 (31) | 32 (31) |

and were less frequently associated with diarrhea than ventilated improved pit latrines (OR, 0.50 [0.23 to 1.09]; $P = 0.082$).

There was not a significant relationship between the risk of having a case of disease and birthweight (OR, 0.85 [0.56 to 1.29]; $P = 0.46$), religion (OR, 1.01 [0.62 to 1.64]; $P = 0.96$); having been in “shamba” (agricultural garden outside of town) in the last 7 days (OR, 1.6 [0.86 to 2.94]; $P = 0.14$); having a career education (OR, 1.05 [0.96 to 1.15]; $P = 0.26$); type of house (OR, 1.30 [0.77 to 2.16]; $P = 0.33$); iron roof (OR 1.35 [0.82 to 2.22]; $P = 0.24$); type of water source (compared with tap water, the covered well has an OR of 0.60 [0.30 to 1.21], the uncovered well has an OR of 0.48 [0.81 to 2.68], and the river has an OR of 1.21 [0.70 to 21.04] [$P = 0.51$]), boiling drinking water (OR, 0.67 [0.25 to 1.80]; $P = 0.43$); filtering drinking water (OR, 1 [0.25 to 3.40]; $P = 1$); or having a toilet cover (OR, 1.25 [0.71 to 2.18]; $P = 0.43$).

In a multivariate analysis, the number of siblings, the number of siblings alive, the distance to the water source, and having a latrine at home were independently related to the risk of diarrhea (Table 2).

Microbiological studies. In the crude analysis, the only pathogen significantly associated with diarrhea was *Shigella* species

TABLE 2. Isolated enteropathogens and the risk of diarrhea

| Enteropathogen(s) | Unadjusted (n = 309) | | | Adjusted (n = 303) ^a | | |
|-----------------------------------|-------------------------|---------------------|-------|------------------------------------|------------|-------|
| | OR ^b | 95% CI ^c | P | OR ^b | 95% CI | P |
| <i>G. lamblia</i> | 0.93 | 0.47–1.81 | 0.820 | 1.06 | 0.51–2.19 | 0.878 |
| <i>G. lamblia</i> (trophozoites) | 1.90 | 0.85–4.26 | 0.122 | 1.82 | 0.76–4.34 | 1.80 |
| <i>E. histolytica</i> | + | | 0.036 | + | | 0.026 |
| <i>Cryptosporidium</i> | + | | 0.138 | + | | 0.054 |
| <i>S. flexneri</i> | 3.00 | 1.23–7.34 | 0.015 | 2.86 | 1.07–7.64 | 0.033 |
| <i>S. sonnei</i> | + | | 0.138 | + | | 0.543 |
| <i>Shigella</i> (all) | 3.25 | 1.35–7.84 | 0.008 | 2.90 | 1.10–7.68 | 0.029 |
| <i>Salmonella</i> | 0 | | 0.368 | 0 | | 0.503 |
| rotavirus | + | | 0.003 | + | | 0.003 |
| ETEC (LT ⁺) | 1.40 | 0.53–3.68 | 0.500 | 1.52 | 0.49–4.72 | 0.472 |
| ETEC (ST ⁺) | 1.32 | 0.60–2.91 | 0.493 | 1.86 | 0.80–4.34 | 0.156 |
| ETEC (LT ⁺ only) | 1.25 | 0.41–3.82 | 0.698 | 1.12 | 0.30–1.24 | 0.863 |
| ETEC (ST ⁺ only) | 1.22 | 0.51–2.90 | 0.654 | 1.66 | 0.66–4.16 | 0.283 |
| ETEC (LT/ST ⁺) | 2.00 | 0.28–14.20 | 0.492 | 3.30 | 0.40–27.07 | 0.275 |
| EAggEC | 0.91 | 0.43–1.93 | 0.799 | 0.75 | 0.33–1.72 | 0.491 |
| <i>E. coli</i> EAST1 ⁺ | 1.06 | 0.60–1.86 | 0.848 | 1.30 | 0.71–2.40 | 0.397 |
| EPEC | 0.57 | 0.12–2.75 | 0.464 | 0.78 | 0.15–4.18 | 0.769 |

^a By conditioned logistic regression adjusted by number of siblings, number of siblings alive, distance to water source, and having a latrine at home.

^b OR is reported as + (infinite) or 0 are not properly estimated because of an insufficient enough number of positives. Such results should be interpreted with caution.

^c CI, confidence interval

(13 isolates from patients, 8 from controls); OR = 3; $P < 0.01$). Twelve of the 13 (92.3%) isolates were *Shigella flexneri*, the other isolate being *Shigella sonnei*. Other pathogens isolated (number of patients/controls, respectively) were salmonella (0/1), LT ETEC (5/8), ST ETEC (9/15), ST-LT ETEC (2/2), EAggEC (11/24), EPEC (4/7), rotavirus (4/0), *Giardia lamblia* (15/32), *Entamoeba histolytica* (2/0), *Cryptosporidium* sp. (1/0), *Isospora belli* (0/1), *Ascaris lumbricoides* (0/1), hookworm (0/5), *Strongyloides stercoralis* (0/2). In 38 (37.25%) patients and 96 (46.76%) controls no enteropathogen was isolated ($P = 0.58$ and $P = 0.043$, respectively). *Campylobacter*, verotoxigenic and enterohemorrhagic *E. coli* and *Yersinia* strains were not isolated in any case.

In 64 *E. coli* strains EAST1 was detected. Twenty-two strains with EAST1 were isolated from patients (21.35%) and 42 strains were isolated from controls (20.38%). Four EAggEC strains isolated from patients and four EAggEC strains from controls were EAST1 positive.

In Table 2 the isolated enteropathogens and the risk of diarrhea, adjusted by number of siblings, number of siblings alive, distance to the water source, and having a toilet at home, are shown.

Dehydration was detected in 11 patients (2 with *Shigella*, 2 with rotavirus plus EAST1, 1 with ST ETEC, 1 with LT ETEC plus *G. lamblia*, and 1 with EAST1). In four patients no enteropathogen was isolated. None of the EAST1-positive *E. coli* strains isolated were EAggEC.

DISCUSSION

The finding that children born earlier in a family were less likely to have cases of disease than children born later may be a reflection of changing patterns of maternal care with successive children. The decrease in risk of diarrhea with increasing number of siblings, especially when more of the siblings survived, was a dominant finding and suggests that being part of a large family reduces the risk of diarrhea. One possible explanation may be related to maternal experience affecting hygiene

practices. At variance with other publications on the same subject (8), our study was unable to show that breast-feeding was a protective factor.

Although it might be anticipated that children with a latrine on their compound may be less likely to develop diarrhea, we were unable to demonstrate a protective effect of ventilated improved pit latrines over the simple pit type. Increasing distance from the water source may be associated with a reduced risk of diarrhea by reducing exposure to contaminated water.

There were high rates of positive stool samples in children without diarrhea (52.24%), underlining the difficulties of determining the cause of an episode of diarrhea. *Shigella* was the only enteropathogen significantly associated with the rise of diarrhea. Other well known enteropathogens such as *G. lamblia*, rotavirus, or ETEC strains as well as EAggEC strains were with no statistical significance.

EAggEC strains were first described as a putative virulence group by Nataro et al. (17). In some studies, however, EAggEC was not related to diarrhea (1, 10). Even in studies where EAggEC strains were associated statistically with diarrhea, the percentage of controls with this enteropathogen were high (31% of controls versus 68% of patients with persistent diarrhea) (7). This fact suggests that children in these settings are frequently exposed to these enteropathogens. In Ifakara the rate of isolation is lower than that in Brazil and is similar in cases and controls (10.6% and 11.6%, respectively). This fact suggests that EAggEC strains are not related to diarrhea in this setting or that these potential enteropathogens may be carried by asymptomatic people in a high percentage as Black et al. found in Peru (4) for other enteropathogens. Some authors have suggested that host factors like vertically acquired antibodies or breast milk could play a role in the pathogenesis of persistent (21) or acute (16) diarrhea. Other factors incriminated are related to the lack of virulence factors from several lines of some enteropathogens, as described for *Campylobacter* species (12) or *G. lamblia* (9). Another hypothesis could be an unexpectedly long period of EAggEC strain excretion in feces after a diarrhea episode treated with only rehydration salts and exceeding the 2 weeks without diarrhea required for controls. Acquired immunity from previous infections at an earlier age could be another explanation, but the greatest number of EAggEC strains isolated from controls was in children less than 1 year old.

ETEC is a well known enteropathogen. Together with rotavirus, ETEC strains cause an important number of cases of diarrhea illness with dehydration (3). However, in some studies ETEC strains were not related to diarrheal illness (4, 16). However, for the ST ETEC specimens there is a difference between the crude and adjusted analysis, probably due to the loss of controls in the adjusted analysis. Thus, results should be interpreted with caution. Besides the same circumstances mentioned for EAggEC strains, other possibilities are that frequent subclinical infections or the long persistent infection after a short and limited diarrheal episode (4). The reasons for this could be ingestion of an insufficient number of organisms to cause disease or acquired or passive immunity acquired from breast milk feeding. However, the similar association with diarrhea among different ages for these enteropathogens provides no clear evidence for an important role of either passive or active immunity.

We could have similar conclusions about *Campylobacter*, EPEC, and *Giardia* strains, for which several studies provide a disparity of results and a high level of asymptomatic carriers (4, 6, 19). However, and in contrast with other studies (16), the low rate of isolation of EAF-positive EPEC strains as well as *bfp* and *eae*-positive EPEC strains is highly surprising. In this

study, *Shigella* spp. are the only enteropathogen statistically related with diarrhea.

We cannot provide conclusions about rotavirus because of the low rate of detection of this virus. On the other hand, this result is not surprising because usually during the rainy season the percentages of diarrhea due to rotavirus are lower than during the dry season (11). A high prevalence *Campylobacter* is described during the wet season (5), although other authors did not find seasonal differences (14). Despite Lindblom et al. (14), who described a high percentage of *Campylobacter* isolates in children from Tanzania, our results show a different pattern—probably because in our study, few children were less than 12 months old, an age at which *Campylobacter* is more prevalent.

Intestinal nematodes do not seem to play a role in diarrheal diseases in this area. The low rates of nematodes isolated from patients and controls suggest a low incidence of these health problems in the Ifakara region for patients at this age.

In summary, no significant differences between patients and controls were observed for the enteropathogens causing diarrhea in children under 5 of age in Ifakara, Tanzania, except for *Shigella*. The above-mentioned hypothesis and, above all, the possible role of acquired immunity, can provide some explanations for these results, although further studies are necessary to elucidate it.

Shigella was the only species of enteropathogen significantly associated with an increased risk of diarrhea in children under 5 years of age in Ifakara, Tanzania. Asymptomatic infection was common and may have arisen due to tolerance-inducing immune mechanisms, intraspecies variation in virulence, or prolonged excretion of organisms after a diarrheal illness. Whatever the reason, it is sobering to realize that the absence of controls in this study would have led to a very different interpretation of the results for these patients.

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